Figure S1. Similar to a Reduction in Raptor Expression, a Reduction in DEPTOR Expression Protects Cells from Apoptosis Induced by Serum Withdrawal.

HT-29 cells expressing shRNAs targeting DEPTOR, raptor, or luciferase were serum starved for 6 hours. Cell lysates were analyzed by immunoblotting for the levels of indicated proteins and phosphorylation states.

Figure S2. PTEN Positively Regulates DEPTOR mRNA Expression.

(A) PTEN Loss Reduces DEPTOR mRNA Expression. HeLa, PC3, and U87 cells were seeded at equal density. 48 hours after seeding DEPTOR mRNA was determined by qRT-PCR from total RNA samples and normalized to GADPH mRNA levels. Error bars indicate mean ± standard deviation for n=3 per condition.

(B) Expression of an shRNA targeting PTEN in HeLa cells reduces DEPTOR mRNA expression. Five days after transductions with an shRNA-expressing lentivirus, cells were lysed and analyzed by immunoblotting for PTEN levels. DEPTOR mRNA was prepared and analyzed as in (A).

Figure S3. Further Characterization of DEPTOR Phosphorylation and its Functions.

(A) Schematic representation of the location of phosphorylation sites identified in DEPTOR by mass spectrometry. All 13 phosphorylation sites were mutated to alanine as described in the methods.

(B) DEPTOR gel mobility is increased by Calf Intestinal Phosphatase (CIP) treatment. Immunoprecipitated wild-type recombinant DEPTOR was incubated without CIP or with active or heat-inactivated CIP for 30 minutes and analyzed by SDS-PAGE followed by immunoblotting.

(C) mTOR inhibitors decrease the recognition of DEPTOR by a phospho-specific antibody. Serum-replete HEK-293T cells expressing FLAG-DEPTOR were treated with vehicle, 50 nM rapamycin, or 250 nM Torin1 for 16 hours. Immunoblotting was performed for the indicated proteins and phosphorylation states. A phospho-specific antibody designed against phospho-S877 raptor cross-reacts with DEPTOR and was used to detect phospho-DEPTOR.
(D) Proline-directed DEPTOR phosphorylation sites serines 244, 265, 293, and 299 are dephosphorylated in the presence of Torin1. Serum-replete HEK-293T cells expressing FLAG-DEPTOR were treated with vehicle or 250nM Torin1 for 16 hours. After immunopurification from cell lysates, the extent of phosphorylation at each of the indicated sites was measured using mass spectrometry as described in the methods.
(E) Elimination of the DEPTOR phosphorylation sites impairs the serum-induced mobility shift seen in SDS-PAGE analyses of wild-type DEPTOR. HeLa cells were transfected with 50 ng of the indicated plasmids expressing FLAG-DEPTOR. Three days later, cells were starved for serum for 30 hours and, where indicated, stimulated with serum for the specified times. Cell lysates were analyzed by immunoblotting for levels of recombinant DEPTOR and, as a loading control, mTOR. Arrows indicate serum-induced mobility shifts of wild-type and 13xS/T->A mutant FLAG-DEPTOR.
(F) Overexpression of recombinant wild-type DEPTOR blocks serum-induced degradation of recombinant and endogenous DEPTOR. Myc-tagged DEPTOR was induced by treatment with 10 ng/ml doxycycline in Tet-On HeLa cells. Two days post-induction, non-induced, and induced cells were serum starved for 30 hours and stimulated with serum for the specified times. Cell lysates were analyzed by immunoblotting for the levels of the indicated proteins.

Figure S4. Overexpression of DEPTOR Activates Phosphorylation of T308 and S473 of Akt1 in a Dose-Dependent Manner.

p53-/- MEFs cells were cotransfected with expression plasmids encoding HA-GST-Akt1 (200 ng) as well as 200 ng or 2 μg of the indicated FLAG-tagged

proteins. Cell lysates were prepared 24 hours after transfection and were analyzed by immunoblotting for the levels of the indicated proteins and phosphorylation states.

Figure S5. Effects of Prolonged Inhibition of mTOR with the ATP-Competitive Inhibitor PP242 on mTORC1 and PI3K/mTORC2/Akt Signaling.
HeLa cells were treated with the specified concentrations of PP242 or vehicle for either 1 hour or 48 hours. Cell lysates were prepared and analyzed by immunoblotting for the levels of the indicated proteins and phosphorylation states.

Figure S6. DEPTOR Expression across Human Cancer Cell lines Anti-Correlates with Cell Size.
(A) Indicated cell lines were seeded at equal density and 48 hours later cell lysates were prepared and analyzed by immunoblotting for DEPTOR. mTOR expression was used as a loading control. Multiple Myeloma cell lines with or without c-MAF/MAFB translocations are indicated where known.
(B) DEPTOR mRNA levels anti-correlate with cell size across a variety of human cell lines. Mean values of DEPTOR mRNA levels and cell diameter are shown in Table S1. Cell size was measured using a Coulter Counter. Non-Multiple Myeloma cell lines (PC3, HEK-293T, and HeLa) are indicated by red squares. Multiple Myeloma cell lines are indicated by black squares.

Figure S7. A Reduction in DEPTOR Expression in 8226 Cells Activates the In Vitro Kinase Activity of mTORC2 Despite Decreasing Akt S473 Phosphorylation in Cells.
8226 cells were infected with lentiviruses expressing shRNAs targeting GFP or DEPTOR. Six days post-infection, mTOR immunoprecipitates were prepared from cell lysates (0.2 mg total protein) and analyzed for mTORC1/2 kinase activities toward S6K1 and Akt1 and for levels of mTOR and DEPTOR.

Figure S8. A Reduction in TSC2 Expression Increases mTORC1 Signaling but Represses PDGFR Protein Levels.
OCI-MY5 cells were infected with a control shRNA or an shRNA targeting TSC2. Five days after infection, cell lysates were analyzed for the indicated protein levels and phosphorylation states.

Figure S9. A Raptor Knockdown Restores PI3K Signaling in 8226 Cells with a DEPTOR knockdown. 8226 cells co-expressing shRNAs targeting luciferase or DEPTOR along with shRNAs targeting luciferase or raptor were lysed five days after infection and cell lysates were analyzed for the indicated protein levels and phosphorylation states.

Table S1. DEPTOR mRNA Expression Analysis in Human Cancers
(A) Figure 6A worksheet. Listing of primary references used to generate meta-analysis of DEPTOR mRNA expression in human cancers.
(B) Figure 6B worksheet. Raw data for DEPTOR and housekeeping mRNA expression in MM tumor samples and plasma cells.
(C) Figure S6B worksheet. Raw data for DEPTOR mRNA expression and cell size measurements in Multiple Myeloma cell lines.